

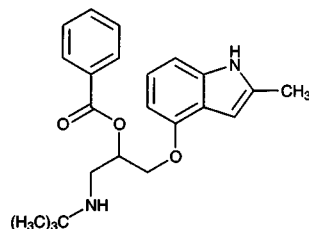
# Bopindolol

**Molecular formula:** C<sub>23</sub>H<sub>28</sub>N<sub>2</sub>O<sub>3</sub>

**Molecular weight:** 380.49

**CAS Registry No.:** 62658-63-3

**Merck Index:** 1362



## SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 50  $\mu$ L 80 ng/mL mepindolol in 100 mM acetic acid + 100  $\mu$ L 1 M NaOH, vortex for 10 s, centrifuge at 1400 g for 4 min, add the supernatant to a 1 mL Extrelut R SPE cartridge, wait for 15 min, elute with 6 mL heptane:isoamyl alcohol 95:5. Add 200  $\mu$ L 100 mM acetic acid to the eluate, shake for 15 min, centrifuge at 1400 g for 5 min, remove the aqueous phase and keep it at 4°, inject a 100  $\mu$ L aliquot of the aqueous phase.

## HPLC VARIABLES

**Column:** 83  $\times$  4.6 3  $\mu$ m Nucleosil ODS

**Mobile phase:** MeOH:30 mM pH 2.2 KH<sub>2</sub>PO<sub>4</sub> 30:70

**Column temperature:** 40

**Flow rate:** 1

**Injection volume:** 100

**Detector:** E, ESA Model 5100 A, 5020 guard cell at +1 V (between pump and injector), 5011 dual analytical cell +160 and +450 mV, +450 mV cell monitored

## CHROMATOGRAM

**Retention time:** 4.7 (for bopindolol hydrolysis product, 4-(2-hydroxy-3-tert-butylaminopropyl)-2-methylindole)

**Internal standard:** mepindolol (3.3) (structure shown is 3-methylpindolol, not 2-methylpindolol=mepindolol)

**Limit of detection:** 0.05 ng/mL

## OTHER SUBSTANCES

**Noninterfering:** digoxin, phenprocoumon, chlorthalidone

## KEY WORDS

plasma; SPE; pharmacokinetics

## REFERENCE

Humbert,H.; Denouel,J.; Keller,H.P. Column liquid chromatographic determination of hydrolysed bopindolol, in the picogram per millilitre range in plasma, using cartridge extraction and dual electrochemical detection, *J.Chromatogr.*, **1987**, 422, 205–215.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 70  $\mu$ L 1 M NaOH + 25  $\mu$ L 83.2 ng/mL IS in 100 mM acetic acid, vortex for 5 s, add 5 mL heptane:isoamyl alcohol 95:5, shake for 15 min on a horizontal shaker, centrifuge at 1400 g for 10 min. Remove 4.2 mL of the organic phase and add it to 150  $\mu$ L 100 mM acetic acid, shake for 15 min on a horizontal shaker, centrifuge at 1400 g for 10 min, inject a 50  $\mu$ L aliquot of the aqueous phase.

## HPLC VARIABLES

**Guard column:** 15  $\times$  3.2 7  $\mu$ m Pierce RP-18

**Column:** 150  $\times$  4.6 5  $\mu$ m Ultrasphere ODS C18

**Mobile phase:** MeOH:30 mM potassium phosphate, adjusted to pH 2.2 with phosphoric acid

**Flow rate:** 1

**Injection volume:** 50

**Detector:** E, ESA Coulochem Model 5100A, model 5010 analytical cells, detector 1 +150 mV, detector 2 +550 mV, monitor cell 2

---

### CHROMATOGRAM

**Retention time:** 14 (as hydrolysis product)

**Internal standard:** 2-hydroxy-N-isopropyl-3-(3-methyl-1H-indol-4-yloxy)-1-propylamine (17-895, 3-methylpindolol) (9)

**Limit of detection:** 250 ng/mL

---

### OTHER SUBSTANCES

**Noninterfering:** allopurinol, amphogel, aspirin, azathioprine, calcitonin, calcitriol, captopril, cimetidine, cloxacillin, cyclosporine, digoxin, diltiazem, ergoloid, furosemide, glyburide, loperamide, nifedipine, nitroglycerin, prazosin, prednisone, propranolol, sulfinpyrazone, tolbutamide

---

### KEY WORDS

plasma

---

### REFERENCE

Perkins,S.L.; Tatttrie,B.; Johnson,P.M.; Rabin,E.Z. Analytical problems encountered during high-performance liquid chromatographic separation and coulometric detection of bopindolol metabolites in human plasma, *Ther.Drug Monit.*, **1988**, 10, 480–485.

---

### SAMPLE

**Matrix:** solutions

---

### HPLC VARIABLES

**Column:** 300 × 3.9 5 μm Nova-Pak C18

**Mobile phase:** MeOH:buffer 50:50 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 4 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)

**Column temperature:** 30

**Flow rate:** 1

**Injection volume:** 100

**Detector:** UV 220

---

### CHROMATOGRAM

**Retention time:** k' 5.35

---

### OTHER SUBSTANCES

**Also analyzed:** alprenolol, betaxolol, propranolol, tertatolol

---

### REFERENCE

Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, 32, 14–20.

---

### SAMPLE

**Matrix:** solutions

---

### HPLC VARIABLES

**Column:** 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)

**Mobile phase:** MeCN:100 mM pH 7.0 phosphate buffer 20:80

**Flow rate:** 1**Detector:** UV 254

---

**CHROMATOGRAM****Retention time:** k' 2.86

---

**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bupranolol, carteolol, celiprolol, chlorpyramine, chlorpheniramine, cicloprolol, cimetidine, cinarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotiline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripeleennamine, triprolidine, tymazoline, UK-14,304

---

**REFERENCE**

Kaliszan, R.; Nasal, A.; Turowski, M. Binding site for basic drugs on  $\alpha_1$ -acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed. Chromatogr.*, **1995**, *9*, 211–215.

---

**SAMPLE****Matrix:** urine

**Sample preparation:** 1 mL Urine + 10 mg  $\beta$ -glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

---

**HPLC VARIABLES**

**Column:** A 10  $\times$  4.6 5  $\mu$ m Spherisorb cyanopropyl; B 250  $\times$  4.6 Capcell Pak C18 UG-120 (Shiseido)

**Mobile phase:** A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)

**Flow rate:** A 1.25; B 1

**Injection volume:** 100

**Detector:** UV 220

---

**CHROMATOGRAM****Retention time:** 12.5**Limit of detection:** 250 ng/mL

---

**OTHER SUBSTANCES**

**Extracted:** acebutolol, alprenolol, amphetamine, atenolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, pindolol, propranolol, timolol

---

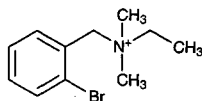
**KEY WORDS**column-switching

---

**REFERENCE**

Saareninen, M.T.; Sirén, H.; Riekkola, M.-L. Screening and determination of  $\beta$ -blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J. Chromatogr. B*, **1995**, *664*, 341–346.

# Bretylium tosylate



**Molecular formula:**  $C_{18}H_{24}BrNO_3S$

**Molecular weight:** 414.4

**CAS Registry No.:** 61-75-6, 59-41-6 (free base)

**Merck Index:** 1395

**Lednicer No.:** 1 55

## SAMPLE

**Matrix:** blood

**Sample preparation:** Condition a Bond-Elut CBA cation-exchange SPE cartridge with 3 mL MeOH, 3 mL water, 1 mL 50 mM pH 9.0 borate. 250  $\mu$ L Plasma + 200  $\mu$ L 1  $\mu$ g/mL D-tubocurarine, add to SPE cartridge, wash with 3 mL water, wash with 1 mL 50 mM pH 3.0  $NaH_2PO_4$ , wash with 1 mL water, wash with two 500  $\mu$ L portions of MeOH, elute with two 500  $\mu$ L portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200  $\mu$ L MeOH:MeCN:water 30:15:55 adjusted to pH 3.4 with 1 M phosphoric acid, inject a 70  $\mu$ L aliquot. (Acidified MeOH was 833  $\mu$ L HCl in 100 mL MeOH.); SPE

## HPLC VARIABLES

**Column:** 100  $\times$  4.9 5  $\mu$ m octyl Spherisorb

**Mobile phase:** MeOH:MeCN:buffer 30:15:55 adjusted to pH 3.4 with 1 M phosphoric acid (Buffer was 10 mM sodium octanesulfonate and 1.5 mM dibutylamine.)

**Flow rate:** 2.5

**Injection volume:** 70

**Detector:** UV 272

## CHROMATOGRAM

**Retention time:** 3

**Internal standard:** D-tubocurarine (5)

**Limit of quantitation:** 625 ng/mL

## KEY WORDS

plasma; SPE; human; pig; pharmacokinetics

## REFERENCE

Théorêt,Y.; Varin,F. Simple, rapid and selective method using high-performance liquid chromatography for the determination of bretylium in plasma, *J.Chromatogr.*, **1992**, 575, 162–166.

---

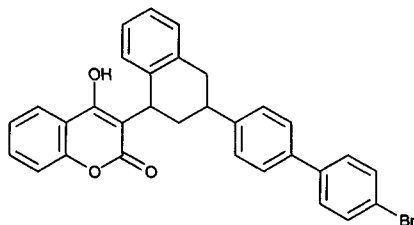
# Brodifacoum

**Molecular formula:** C<sub>31</sub>H<sub>23</sub>BrO<sub>3</sub>

**Molecular weight:** 523.43

**CAS Registry No.:** 56073-10-0

**Merck Index:** 1400



---

## SAMPLE

**Matrix:** blood, tissue

**Sample preparation:** Condition a silica Sep-Pak SPE cartridge also containing 2 g sodium sulfate (?) with 5 mL MeOH and 5 mL cyclohexane. Mix 3 mL blood or crushed tissue with 1 mL 20 µg/mL IS, adjust to pH 3-4 with 0.5 M sulfuric acid, extract three times with 10 mL MeOH:chloroform 10:90 (Caution! Chloroform is a carcinogen!). Evaporate at 40°, re-dissolve the residue in 5 mL cyclohexane, sonicate and centrifuge three times. Remove a 5 mL aliquot of the top layer, evaporate at 40°. Reconstitute the residue in 5 mL cyclohexane. Add to the SPE cartridge, elute with 5 mL MeOH, evaporate at 40°, reconstitute the residue in MeOH, inject an aliquot.

---

## HPLC VARIABLES

**Column:** 200 mm long µBondapak C18

**Mobile phase:** MeOH:0.8% acetic acid 80:20

**Flow rate:** 1

**Injection volume:** 10

**Detector:** UV 280

---

## CHROMATOGRAM

**Retention time:** 17.8

**Internal standard:** N,N-diphenylbenzidine (9.3)

**Limit of detection:** 25 ng/mL

---

## OTHER SUBSTANCES

**Extracted:** bromadiolone, coumarin, coumatetralyl, warfarin

---

## KEY WORDS

SPE; plasma; heart; lung; liver; kidney; spleen

---

## REFERENCE

Park,S.W.; Seo,B.S.; Kim,E.H.; Kim,D.H.; Paeng,K.-J. Purification and determination procedure of coumarin derivatives, *J.Forensic Sci.*, **1996**, *41*, 685-688.

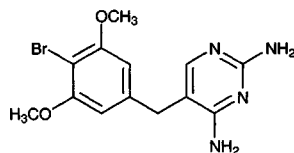
# Brodimoprim

**Molecular formula:** C<sub>13</sub>H<sub>15</sub>BrN<sub>4</sub>O<sub>2</sub>

**Molecular weight:** 339.19

**CAS Registry No.:** 56518-41-3

**Merck Index:** 1401



## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Dilute urine 1:10 with water. 200  $\mu$ L Plasma, blood, or diluted urine + 50  $\mu$ L 5  $\mu$ g/mL IS in MeOH + 500  $\mu$ L 400 mM borate buffer + chloroform, vortex for a few s, centrifuge at 1700 g at 4° for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200  $\mu$ L mobile phase, let stand for at least 1 h at room temperature, inject a 20-50  $\mu$ L aliquot. (Deconjugate 200  $\mu$ L plasma or diluted urine with 100  $\mu$ L 68 U/mL  $\beta$ -glucuronidase in 500 mM pH 5.0 sodium acetate buffer, heat at 37° for 4 h, add 100  $\mu$ L 400 mM pH 10 borate buffer, add 50  $\mu$ L 5  $\mu$ g/mL IS in MeOH, extract as before.) (Use chloroform stabilized with EtOH.)

## HPLC VARIABLES

**Guard column:** 10  $\times$  3 30  $\mu$ m Chrompack guard column

**Column:** 33  $\times$  4.6 3  $\mu$ m Pecosphere-3  $\times$  3 CR C18

**Mobile phase:** MeCN:25 mM phosphate buffer 23:77 + 0.1% triethylamine, adjusted to pH 7.5 with 10 M KOH

**Flow rate:** 1

**Injection volume:** 20-50

**Detector:** F ex 290 em 340

## CHROMATOGRAM

**Retention time:** 1.1

**Internal standard:** 2,4-diamino-5-(4'-(2-propenyl)-3',5'-dimethoxybenzyl)pyrimidine (Ro-11-3296) (4.2)

**Limit of detection:** 5 ng/mL

## OTHER SUBSTANCES

**Extracted:** metabolites

**Simultaneous:** flecainide

**Noninterfering:** trimethoprim,  $\beta$ -blockers

## KEY WORDS

plasma; use UV detection with clonazepam IS and LOD 100 ng/mL

## REFERENCE

Gaspari,F.; Taiocchi,L.; Pochobradsky,M.G. Determination of brodimoprim and its hydroxy metabolite in human plasma, blood and urine by high-performance liquid chromatography, *J. Chromatogr.*, **1992**, 577, 123-128.

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Extract plasma or urine with n-butyl acetate:MeOH 98:2, inject an aliquot of the organic layer.

## HPLC VARIABLES

**Column:** 250  $\times$  4.6 5  $\mu$ m Lichrosorb Si 60

**Mobile phase:** 12.5 mL MeOH + 1.5 mL 33% ammonia made up to 250 mL with 2-pentanone

**Flow rate:** 2.5  
**Injection volume:** 100  
**Detector:** F ex 290 em 340

---

#### CHROMATOGRAM

**Retention time:** 4.3  
**Internal standard:** Ro 11-8958 (3.2)  
**Limit of quantitation:** 40 ng/mL

---

#### KEY WORDS

plasma; normal phase; pharmacokinetics

---

#### REFERENCE

Weidekamm,E. Pharmacokinetics of brodimoprim, *J.Chemother.*, **1993**, 5, 475–479.

---

#### SAMPLE

**Matrix:** cell suspensions  
**Sample preparation:** Cool cell suspension in an ice bath, centrifuge at 800 g at 4° for 15 min, inject an aliquot of the supernatant.

---

#### HPLC VARIABLES

**Column:**  $\mu$ Bondapak C18  
**Mobile phase:** MeCN:MeOH:water 10:30:60 containing 10 mM  $K_2HPO_4$   
**Flow rate:** 2  
**Detector:** UV 280

---

#### CHROMATOGRAM

**Limit of detection:** 250 ng/mL

---

#### OTHER SUBSTANCES

**Also analyzed:** trimethoprim

---

#### REFERENCE

Climax,J.; Lenehan,T.J.; Lambe,R.; Kenny,M.; Caffrey,E.; Darragh,A. Interaction of antimicrobial agents with human peripheral blood leucocytes: uptake and intracellular localization of certain sulphonamides and trimethoprim, *J.Antimicrob.Chemother.*, **1986**, 17, 489–498.

---

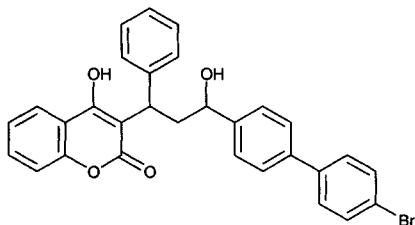
# Bromadiolone

**Molecular formula:** C<sub>30</sub>H<sub>23</sub>BrO<sub>4</sub>

**Molecular weight:** 527.41

**CAS Registry No.:** 28772-56-7

**Merck Index:** 1403



---

## SAMPLE

**Matrix:** blood, tissue

**Sample preparation:** Condition a silica Sep-Pak SPE cartridge also containing 2 g sodium sulfate (?) with 5 mL MeOH and 5 mL cyclohexane. Mix 3 mL blood or crushed tissue with 1 mL 20 µg/mL IS, adjust to pH 3-4 with 0.5 M sulfuric acid, extract three times with 10 mL MeOH:chloroform 10:90 (Caution! Chloroform is a carcinogen!). Evaporate at 40°, re-dissolve the residue in 5 mL cyclohexane, sonicate and centrifuge three times. Remove a 5 mL aliquot of the top layer, evaporate at 40°. Reconstitute the residue in 5 mL cyclohexane. Add to the SPE cartridge, elute with 5 mL MeOH, evaporate at 40°, reconstitute the residue in MeOH, inject an aliquot.

---

## HPLC VARIABLES

**Column:** 200 mm long µBondapak C18

**Mobile phase:** MeOH:0.8% acetic acid 80:20

**Flow rate:** 1

**Injection volume:** 10

**Detector:** UV 280

---

## CHROMATOGRAM

**Retention time:** 7.1

**Internal standard:** N,N-diphenylbenzidine (9.3)

**Limit of detection:** 25 ng/mL

---

## OTHER SUBSTANCES

**Extracted:** brodifacoum, coumarin, coumatetralyl, warfarin

---

## KEY WORDS

SPE; plasma; heart; lung; liver; kidney; spleen

---

## REFERENCE

Park,S.W.; Seo,B.S.; Kim,E.H.; Kim,D.H.; Paeng,K.-J. Purification and determination procedure of coumarin derivatives, *J.Forensic Sci.*, **1996**, *41*, 685-688.



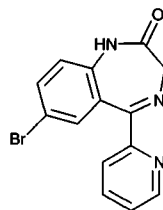
# Bromazepam

**Molecular formula:** C<sub>14</sub>H<sub>10</sub>BrN<sub>3</sub>O

**Molecular weight:** 316.16

**CAS Registry No.:** 1812-30-2

**Merck Index:** 1406



## SAMPLE

**Matrix:** blood

**Sample preparation:** 500  $\mu$ L Serum + 20  $\mu$ L 20  $\mu$ g/mL IS + 200  $\mu$ L 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100  $\mu$ L mobile phase, inject a 20  $\mu$ L aliquot. (Caution! Chloroform is a carcinogen!)

## HPLC VARIABLES

**Column:** 100  $\times$  4.6 2  $\mu$ m TSK gel Super-ODS (A) or 100  $\times$  4.6 5  $\mu$ m Hypersil ODS-C18 (B)

**Mobile phase:** MeCN:5 mM pH 6 NaH<sub>2</sub>PO<sub>4</sub> 45:55

**Flow rate:** 0.65

**Injection volume:** 20

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 6.6 (A), 25.5 (B)

**Internal standard:** diazepam (29.8 (A), 77.5 (B))

**Limit of quantitation:** 5 ng/mL (A)

## OTHER SUBSTANCES

**Extracted:** chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam

**Simultaneous:** alprazolam

**Noninterfering:** barbitol, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

## KEY WORDS

serum

## REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2- $\mu$ m porous microspherical silica gel, *J.Chromatogr.B*, **1996**, 682, 173–178.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100  $\mu$ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50  $\mu$ L aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

---

**HPLC VARIABLES**

**Column:** 300 × 3.9 4 µm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic))  $\text{KH}_2\text{PO}_4$  adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 234

---

**CHROMATOGRAM**

**Retention time:** 3.67

**Limit of detection:** <120 ng/mL

---

**KEY WORDS**

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vandesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thiopropazine; methadone; amoxapine; quinupramine; opi-pramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozone; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nor-triptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

---

**REFERENCE**

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254-262.

---

**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

---

**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 232.2

---

**CHROMATOGRAM**

**Retention time:** 14.732

---

**KEY WORDS**

whole blood

---

**REFERENCE**

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149-163.

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

---

**OTHER SUBSTANCES**

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotriptyline, benzphetamine, berberine, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapson, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mependazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephentoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methylpyrrol, metoprolol, mi-bolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

**REFERENCE**

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233-242.

# Bromhexine

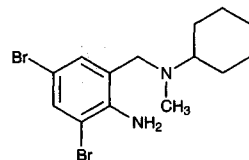
**Molecular formula:** C<sub>14</sub>H<sub>20</sub>Br<sub>2</sub>N<sub>2</sub>

**Molecular weight:** 376.13

**CAS Registry No.:** 3572-43-8, 611-75-6 (HCl)

**Merck Index:** 1412

**Lednicer No.:** 2 96



## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 1.2

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, pen-thienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phen-tolamine, phenylephrine, physyltoloxamine, physostigmine, piminodine, pimezide, pin-dolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piri-tramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolin-tane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, pro-

thipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleennamine, triprolidine, tryptamine, verapamil, xylometazoline

---

## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, 323, 191-225.

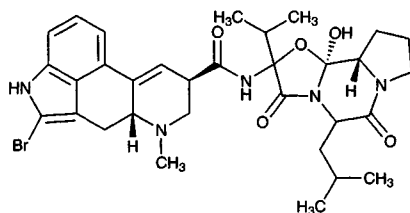
# Bromocriptine

**Molecular formula:** C<sub>32</sub>H<sub>40</sub>BrN<sub>5</sub>O<sub>5</sub>

**Molecular weight: 654.60**

**CAS Registry No.:** 25614-03-3, 22260-51-1 (mesylate)

**Merck Index:** 1437



## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature: 30**

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume: 10-30**

**Detector:** UV 200.5

## CHROMATOGRAM

**Retention time: 16.652**

## KEY WORDS

whole blood

## REFERENCE

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, *763*, 149–163.



# Bromodiphenhydramine

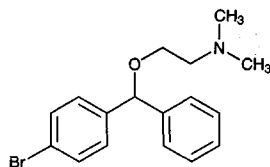
**Molecular formula:** C<sub>17</sub>H<sub>20</sub>BrNO

**Molecular weight:** 334.26

**CAS Registry No.:** 118-23-0, 1808-12-4 (HCl)

**Merck Index:** 1439

**Lednicer No.:** 1 42



## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 3.4

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscyne, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepamine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methyl-ephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphane, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleennamine, triprolidine, tryptamine, verapamil, xylometazoline

---

## REFERENCE

- Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191-225.

# Bromperidol

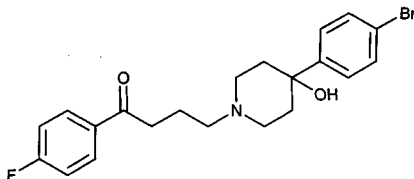
**Molecular formula:**  $C_{21}H_{23}BrFNO_2$

**Molecular weight:** 420.32

**CAS Registry No.:** 10457-90-6

**Merck Index:** 1466

**Lednicer No.:** 2 331



## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10  $\mu\text{g/mL}$  solution in MeOH, inject a 20  $\mu\text{L}$  aliquot.

## HPLC VARIABLES

**Column:** 125  $\times$  4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 2.1

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrizamide, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazoline, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleennamine, triprolidine, tryptamine, verapamil, xylometazoline

---

## REFERENCE

- Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191-225.

# Brompheniramine

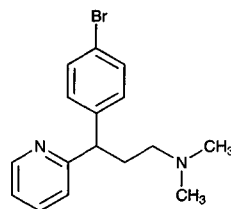
**Molecular formula:** C<sub>16</sub>H<sub>19</sub>BrN<sub>2</sub>

**Molecular weight:** 319.3

**CAS Registry No.:** 86-22-6, 132-21-8 (d-form), 980-71-2 (maleate), 2391-03-9 (d-form maleate)

**Merck Index:** 1467

**Lednicer No.:** 1 77



## SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Serum + 50 µL 0.5 ng/mL (sic) antazoline hydrochloride in water + 5 mL freshly distilled ether, vortex for 1-2 s, add 200 µL 10% KOH, vortex for 15 s, centrifuge for 3-3 min, freeze in MeOH/dry ice. Remove the organic layer and add it to 100 µL 0.5% phosphoric acid, vortex for 15 s, freeze in MeOH/dry ice. Discard the organic layer, remove the last traces of ether with nitrogen for 2-3 min, inject a 100 µL aliquot of the aqueous layer.

## HPLC VARIABLES

**Column:** 5 mm CN Microbond pak radial compression (Waters)

**Mobile phase:** MeCN:buffer 28:72 (Buffer was 75 mM phosphate and 20 mM dibutylamine, adjusted to pH 3.2.)

**Flow rate:** 1

**Injection volume:** 100

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 5.5

**Internal standard:** antazoline hydrochloride (6.3)

**Limit of detection:** 2 ng/mL

## KEY WORDS

serum; pharmacokinetics

## REFERENCE

Simons,F.E.R.; Frith,E.M.; Simons,K.J. The pharmacokinetics and antihistaminic effects of brompheniramine, *J.Allerg.Clin.Immunol.*, **1982**, 70, 458-464.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

## HPLC VARIABLES

**Column:** 300 × 3.9 4 µm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 226

## CHROMATOGRAM

**Retention time:** 7.78

**Limit of detection:** <120 ng/mL

## KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiapropenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindsesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loperazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

## SAMPLE

**Matrix:** blood, tissue

**Sample preparation:** Blood or serum. 1 mL Blood or serum + 1 µg cyanopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 µL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 µL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 µg cyanopramine + 500 µL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for

5 min. Remove the organic layer and add it to 400  $\mu\text{L}$  0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30  $\mu\text{L}$  aliquot of the aqueous layer.

---

**HPLC VARIABLES**

**Guard column:** 15  $\times$  3.2 7  $\mu\text{m}$  RP-18 Newguard (Applied Biosystems)

**Column:** 100  $\times$  4.6 5  $\mu\text{m}$  Brownlee Spheri-5 RP-18

**Mobile phase:** MeCN:100 mM  $\text{NaH}_2\text{PO}_4$ :diethylamine 40:57.5:2.5

**Flow rate:** 2

**Injection volume:** 30

**Detector:** UV 220

---

**CHROMATOGRAM**

**Retention time:** 10.76

**Internal standard:** cianopramine (8.93)

---

**OTHER SUBSTANCES**

**Simultaneous:** amitriptyline, amoxapine, benztropine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, nortriaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranquypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

**Noninterfering:** dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

**Interfering:** doxepin, methadone

---

**KEY WORDS**

serum; whole blood; liver

---

**REFERENCE**

McIntyre, I.M.; King, C.V.; Skafidis, S.; Drummer, O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J. Chromatogr.*, **1993**, 621, 215–223.

---

**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu\text{L}$  MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu\text{L}$  aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

---

**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu\text{m}$  Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10–30

**Detector:** UV 200.5

---

## CHROMATOGRAM

**Retention time:** 13.935

---

## KEY WORDS

whole blood

---

## REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149–163.

---

## SAMPLE

**Matrix:** cell incubations

**Sample preparation:** 40 mL Cell incubation + 50 mL MeOH, shake vigorously for 1 min, centrifuge at 2000 rpm for 10 min, wash the pellet twice with 50 mL portions of MeOH. Combine the supernatants and add 100 mL water, extract three times with 150 mL portions of dichloromethane. Filter the extracts through anhydrous sodium sulfate, evaporate the filtrate to dryness under reduced pressure at 40°, reconstitute with mobile phase, inject an aliquot.

---

## HPLC VARIABLES

**Column:** 250 × 4.6 5 µm cyano-propyl (Beckman)

**Mobile phase:** MeCN:buffer 40:60 (Buffer was 10 mM KH<sub>2</sub>PO<sub>4</sub> containing 20 mM triethylamine, pH 7.0.)

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 254

---

## CHROMATOGRAM

**Retention time:** 9.5

---

## OTHER SUBSTANCES

**Extracted:** metabolites

**Interfering:** chlorpheniramine, pheniramine

---

## KEY WORDS

also semipreparative details

---

## REFERENCE

Hansen,E.B.,Jr.; Cho,B.P.; Korfmacher,W.A.; Cerniglia,C.E. Fungal transformations of antihistamines: metabolism of brompheniramine, chlorpheniramine, and pheniramine to *N*-oxide and *N*-demethylated metabolites by the fungus *Cunninghamella elegans*, *Xenobiotica*, **1995**, 25, 1081–1092.

---

## SAMPLE

**Matrix:** formulations, urine

**Sample preparation:** Tablets. Crush tablets, add 100 mL water and 30-40 mL MeCN, dissolve, add *N,N*-dimethylbenzylamine, make up to 250 or 500 mL with water, centrifuge an aliquot, inject a 20 µL aliquot of the supernatant. Urine. Inject a 100 µL aliquot of urine directly.

---

## HPLC VARIABLES

**Column:** 150 × 4.6 Asahipak ODP-50 C18

**Mobile phase:** MeCN:200 mM pH 7.0 phosphate buffer 27:73

**Flow rate:** 0.8

**Injection volume:** 20-100



**Detector:** Chemiluminescence following post-column reaction. Oxidize a 1 mM tris(2,2'-bipyridine) ruthenium(II) hexachloride solution in 50 mM pH 5.5 acetate buffer to Ru(III) using a Princeton Applied Research polarographic analyzer with a platinum gauze working electrode, platinum wire auxiliary electrode, and a silver wire reference electrode, +950 mV. Pump the reagent solution at 0.28 mL/min and mix with the column effluent, allow to flow through detector. The chemiluminescence detector was a fluorescence detector with the light source removed.

---

#### CHROMATOGRAM

**Retention time:** 8

**Internal standard:** N,N-dimethylbenzylamine

**Limit of detection:** 140 ng/mL

---

#### OTHER SUBSTANCES

**Simultaneous:** pheniramine, chlorpheniramine, pyrilamine, diphenhydramine

---

#### KEY WORDS

tablets

---

#### REFERENCE

Holeman, J.A.; Danielson, N.D. Liquid chromatography of antihistamines using post-column tris(2, 2'-bipyridine) ruthenium(III) chemiluminescence detection, *J.Chromatogr.A*, **1994**, 679, 277-284.

---

#### SAMPLE

**Matrix:** incubations

**Sample preparation:** Extract incubation mixture with four volumes of cold dichloromethane for 3 min, centrifuge at 1000 g for 5 min. Remove the organic layer and pass it through a nylon filter, evaporate the filtrate to dryness, reconstitute the residue in 500  $\mu$ L MeOH, inject a 15  $\mu$ L aliquot.

---

#### HPLC VARIABLES

**Column:** 250  $\times$  4.5 5  $\mu$ m AXXIOM silica (Richard Scientific)

**Mobile phase:** MeCN:MeOH:60% aqueous perchloric acid 60:40:0.08

**Flow rate:** 1

**Injection volume:** 15

**Detector:** UV 260

---

#### CHROMATOGRAM

**Retention time:** 6.4

**Limit of detection:** 5-20 ng/mL

---

#### OTHER SUBSTANCES

**Extracted:** chlorpheniramine N-oxide, chlorpheniramine N-oxide

**Interfering:** brompheniramine

---

#### KEY WORDS

ion-pair; desorption; chromatography; incubations

---

#### REFERENCE

Cashman, J.R.; Yang, Z.-C. Analysis of amine metabolites by high-performance liquid chromatography on silica gel with a non-aqueous ionic eluent, *J.Chromatogr.*, **1990**, 532, 405-410.

---

#### SAMPLE

**Matrix:** solutions

---

#### HPLC VARIABLES

**Column:** 250  $\times$  4 ODS (Hitachi)

**Mobile phase:** MeCN:50 mM phosphoric acid 40:60 containing 300 mM KCl and 300 mM ammonium chloride  
**Column temperature:** 55  
**Flow rate:** 0.6  
**Injection volume:** 20  
**Detector:** UV 265

---

## REFERENCE

Sugawara, M.; Takekuma, Y.; Yamada, H.; Kobayashi, M.; Iseki, K.; Miyazaki, K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J. Pharm. Sci.*, **1998**, *87*, 960–966.

---

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

---

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

---

## CHROMATOGRAM

**Retention time:** 4.4

---

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclimine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenylglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxylbenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen,

procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191-225.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

## HPLC VARIABLES

**Column:** 300 × 3.9 10 µm µBondapak C18

**Mobile phase:** MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 9

## OTHER SUBSTANCES

**Simultaneous:** thonzylamine, pheniramine, tripeleppamine, chlorpheniramine, phenindamine, phenyltoxamine, clemizole

## REFERENCE

Roos, R. W.; Lau-Cam, C. A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J. Chromatogr.*, **1986**, 370, 403-418.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Inject 5 mg ± brompheniramine maleate onto analytical system. After separation on the analytical column use valve switching to collect the enantiomers on two 100 × 10 recovery columns of 12-20 µm Hamilton PRP-1 styrene-divinylbenzene columns. When sufficient material has been collected (60 mg), flush the recovery columns with 180 mL MeOH:water 5:95, and elute the enantiomers with MeOH (analytical column out of circuit). Each enantiomer can be purified by a second pass through the system.

## HPLC VARIABLES

**Column:** 250 × 10 5 µm Spherisorb S5CN cyanopropyl

**Mobile phase:** MeOH:buffer 5:95 containing 12 mg/mL β-cyclodextrin hydrate (Buffer was 0.85% triethylamine adjusted to pH 4 with acetic acid.)

**Flow rate:** 3.5

**Injection volume:** 100

**Detector:** UV 285

## CHROMATOGRAM

**Retention time:** 5.5 (+), 6.5 (-)

---

**KEY WORDS**

semi-preparative; chiral

---

**REFERENCE**

Cooper, A.D.; Jefferies, T.M. Semi-preparative high-performance liquid chromatographic resolution of brompheniramine enantiomers using  $\beta$ -cyclodextrin in the mobile phase, *J. Chromatogr.*, **1993**, 637, 137-143.

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

---

**OTHER SUBSTANCES**

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amyllocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbomal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisolone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystiril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephénytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypyrrolon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, re-

serpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopolin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethiodole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

## REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 Chirex 3020 (Phenomenex)

**Mobile phase:** Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 60:35:5 (EtOH/trifluoroacetic acid was premixed 20:1.)

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 264

## CHROMATOGRAM

**Retention time:** 12, 13 (enantiomers)

## OTHER SUBSTANCES

**Simultaneous:** promethazine

## KEY WORDS

chiral

## REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4 5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

## CHROMATOGRAM

**Retention time:** 11.10 (A), 5.44 (B)

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepox-

ide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfapyrazole, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

---

## KEY WORDS

also details of plasma extraction

---

## REFERENCE

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103–119.

# Brotizolam

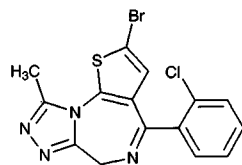
**Molecular formula:** C<sub>15</sub>H<sub>10</sub>BrClN<sub>4</sub>S

**Molecular weight:** 393.69

**CAS Registry No.:** 57801-81-7

**Merck Index:** 1472

**Lednicer No.:** 4 219



## SAMPLE

**Matrix:** bile, blood, urine

**Sample preparation:** Urine, bile. Lyophilize, dissolve in 100 mM pH 5 sodium/potassium buffer, incubate with  $\beta$ -glucuronidase/sulfatase at 37° for 16 h, extract with ether. Plasma. Lyophilize, extract with MeOH.

## HPLC VARIABLES

**Guard column:** 50 × 4.6 30-40  $\mu$ m Lichrosorb RP18

**Column:** 250 × 4.6 10  $\mu$ m Lichrosorb RP18

**Mobile phase:** MeCN:10 mM ammonium carbamate:diethylamine 35:65:0.1 (urine, bile) or MeOH:10 mM ammonium carbamate:diethylamine 45:55:0.1 (plasma)

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 23 (urine, bile), 39 (plasma)

## OTHER SUBSTANCES

**Extracted:** metabolites

## KEY WORDS

plasma; mouse; pharmacokinetics

## REFERENCE

Bechtel, W.D. Blood level, metabolism and excretion of [<sup>14</sup>C]-brotizolam in mice, *Eur.J.Drug Metab.Pharmacokinet.*, **1990**, *15*, 287-293.

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Wash a C2 Bond-Elut SPE cartridge with 1 column volume methanol and 1 column volume buffer. Add 1 mL of urine buffered with pH 6 100 mM phosphate buffer or plasma buffered with pH 8 100 mM phosphate buffer to the SPE cartridge, wash with 3 column volumes of water, wash with 1 mL of MeOH:water 30:70, elute with 1 mL of MeOH:water 60:40. Evaporate the eluate to dryness and take up the residue in 200  $\mu$ L mobile phase, inject an aliquot.

## HPLC VARIABLES

**Column:** 35 × 4.6 5  $\mu$ m ultrabase C18 (Scharlau)

**Mobile phase:** MeOH:water 60:40

**Flow rate:** 0.9

**Injection volume:** 20

**Detector:** UV 240

## CHROMATOGRAM

**Internal standard:** Prazepam

**Limit of detection:** 76 ng/mL

---

**OTHER SUBSTANCES**

**Also analyzed:** diazepam, oxazepam, nordazepam, temazepam, adinazolam, midazolam

---

**KEY WORDS**

plasma; SPE.

---

**REFERENCE**

Casas,M.; Berrueta,L.A.; Gallo,B.; Vicente,F. Solid-phase extraction of 1,4-benzodiazepines from biological fluids, *J.Pharm.Biomed.Anal.*, **1993**, 11, 277–284.

---

---

**SAMPLE**

**Matrix:** microsomal incubations

**Sample preparation:** Mix 200  $\mu\text{L}$  microsomal incubation with 5  $\mu\text{L}$  70% perchloric acid, cool on ice, add 5  $\mu\text{L}$  100  $\mu\text{M}$  diazepam, centrifuge at 15000 g for 2 min. Transfer 170  $\mu\text{L}$  supernatant to another tube and mix with 50  $\mu\text{L}$  1 M MOPS and 5  $\mu\text{L}$  saturated KOH to adjust pH 7.0, centrifuge, inject a 50  $\mu\text{L}$  aliquot of the supernatant.

---

**HPLC VARIABLES**

**Column:** 125  $\times$  4.5  $\mu\text{m}$  Superspher RP-Select B (Merck)

**Mobile phase:** MeOH:MeCN:50mM ammonium acetate 50:5:45

**Column temperature:** 40

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 240

---

**CHROMATOGRAM**

**Retention time:** 5.2

**Internal standard:** diazepam (7.2)

**Limit of quantitation:** 100 nM

---

**OTHER SUBSTANCES**

**Extracted:** metabolites,  $\alpha$ -hydroxy-brotizolam, 6-hydroxy-brotizolam KW human, liver

---

**REFERENCE**

Senda,C.; Kishimoto,W.; Sakai,K.; Nagakura,A.; Igarashi,T. Identification of human cytochrome P450 isoforms involved in the metabolism of brotizolam, *Xenobiotica*, **1997**, 27, 913–922.

---

---

**SAMPLE**

**Matrix:** solutions

---

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu\text{m}$  Supelcosil LC-DP (A) or 250  $\times$  4.5  $\mu\text{m}$  LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

---

**CHROMATOGRAM**

**Retention time:** 7.43 (A), 7.90 (B)



---

**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfipyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

---

**KEY WORDS**

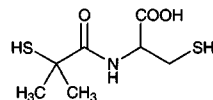
also details of plasma extraction

---

**REFERENCE**

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103–119.

# Bucillamine



**Molecular formula:** C<sub>7</sub>H<sub>13</sub>NO<sub>3</sub>S<sub>2</sub>

**Molecular weight:** 223.32

**CAS Registry No.:** 65002-17-7

**Merck Index:** 1481

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Add 1.05-3 equivalents 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-isothiocyanate to 10 mL of a 100 μM solution of the thiol in MeCN:water 50:50 containing 1-3 equivalents triethylamine, vortex briefly, let stand at room temperature for 30 min, dilute with mobile phase, inject a 20 μL aliquot.

## HPLC VARIABLES

**Column:** 150 × 4.6 5 μm TSKgel ODS-80TM (Tosoh)

**Mobile phase:** MeCN:10 mM pH 2.8 potassium phosphate buffer 53:47

**Column temperature:** 40

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 250

## CHROMATOGRAM

**Retention time:** 3.50 (S), 4.07 (R)

## OTHER SUBSTANCES

**Simultaneous:** cysteine, homocysteine

## KEY WORDS

derivatization; chiral

## REFERENCE

Ito,S.; Ota,A.; Yamamoto,K.; Kawashima,Y. Resolution of the enantiomers of thiol compounds by reversed-phase liquid chromatography using chiral derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, *J.Chromatogr.*, **1992**, 626, 187-196.

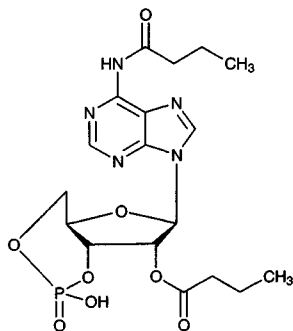
# Bucladesine

**Molecular formula:** C<sub>18</sub>H<sub>24</sub>N<sub>5</sub>O<sub>8</sub>P

**Molecular weight:** 469.39

**CAS Registry No.:** 362-74-3

**Merck Index:** 1483



---

## SAMPLE

**Matrix:** blood

**Sample preparation:** 200 µL Plasma + 200 µL 10% trichloroacetic acid, vortex, cool in ice for 10 min, centrifuge at 3000 rpm for 10 min, inject a 20 µL aliquot of the supernatant.

---

## HPLC VARIABLES

**Column:** 150 × 4 Nucleosil 5C18

**Mobile phase:** MeCN:100 mM KH<sub>2</sub>PO<sub>4</sub>:water 13:2:85

**Flow rate:** 1

**Detector:** UV 273

---

## CHROMATOGRAM

**Limit of detection:** <500 ng/mL

---

## KEY WORDS

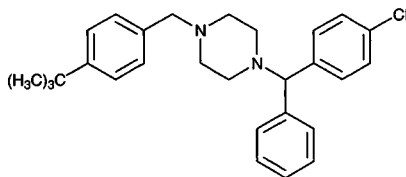
plasma; rat; pharmacokinetics

---

## REFERENCE

Mafune,E.; Takahashi,M.; Takasugi,N. Effect of vehicles on percutaneous absorption of bucladesine (di-butylryl cyclic AMP) in normal and damaged rat skin, *Biol.Pharm.Bull.*, **1995**, *18*, 1539–1543.

# Buclizine



**Molecular formula:**  $C_{28}H_{33}ClN_2$

**Molecular weight:** 433.04

**CAS Registry No.:** 82-95-1, 129-74-8 (HCl)

**Merck Index:** 1484

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 200.5

## CHROMATOGRAM

**Retention time:** 22.752

## KEY WORDS

whole blood

## REFERENCE

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, 763, 149-163.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 1 mg/mL solution in MeOH, inject a 5  $\mu$ L aliquot.

## HPLC VARIABLES

**Column:** 250  $\times$  4.6 5  $\mu$ m Lichrosphere cyanopropyl

**Mobile phase:** Carbon dioxide:MeOH:isopropylamine 90:10:0.05

**Column temperature:** 50

**Flow rate:** 3

**Injection volume:** 5

**Detector:** UV 220

## CHROMATOGRAM

**Retention time:** 1.65

---

**OTHER SUBSTANCES**

**Simultaneous:** benactyzine, hydroxyzine, perphenazine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

---

**KEY WORDS**

SFC; pressure 200 bar

---

**REFERENCE**

Schaeffer, V.H.; Masoud, A.N.; Rubin, R.J. Analysis of monobutyl and dibutyl derivatives of adenosine 3',5'-monophosphate in biological samples using isocratic ion pair high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, 72, 1255-1259.

---

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

---

**HPLC VARIABLES**

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

---

**CHROMATOGRAM**

**Retention time:** 1.4

---

**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclozocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdiazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pi-

zotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

---

## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

---

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 1 mg/mL solution in MeOH, inject a 5  $\mu$ L aliquot.

---

## HPLC VARIABLES

**Column:** 250  $\times$  4.6 5  $\mu$ m Lichrosphere cyanopropyl

**Mobile phase:** Carbon dioxide:MeOH:isopropylamine 90:10:0.05

**Column temperature:** 50

**Flow rate:** 3

**Injection volume:** 5

**Detector:** UV 220

---

## CHROMATOGRAM

**Retention time:** 1.65

---

## OTHER SUBSTANCES

**Simultaneous:** benactyzine, hydroxyzine, perphenazine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

---

## KEY WORDS

SFC; pressure 200 bar

---

## REFERENCE

Berger, T.A.; Wilson, W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

---

## SAMPLE

**Matrix:** solutions

---

## HPLC VARIABLES

**Column:** 250  $\times$  4.6 5  $\mu$ m Vydac 201HS54 C18

**Mobile phase:** Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min

**Flow rate:** 1.5

**Detector:** UV 220 (from Vydac Applications Brochure)

---

## CHROMATOGRAM

**Retention time:** 19

**OTHER SUBSTANCES**

**Simultaneous:** chlorcyclizine, tripeleennamine, triprolidine, methaphenilene, pyrrobutamine, cyclizine, meclizine

---

**REFERENCE**

*Vydac HPLC Catalog, 1994-5,*

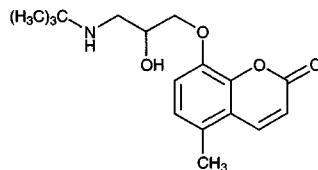
# Bucumolol

**Molecular formula:** C<sub>17</sub>H<sub>23</sub>NO<sub>4</sub>

**Molecular weight:** 305.37

**CAS Registry No.:** 58409-59-9, 80750-21-6 ((±)-form), 30073-40-6 (HCl), 36556-75-9 (HCl (±)-form)

**Merck Index:** 1489



## SAMPLE

**Matrix:** solutions

**Sample preparation:** Mix 100  $\mu$ L of a 10  $\mu$ M solution in MeCN:water:triethylamine 50:50:0.1 with 100  $\mu$ L 1 mM (R)-(-)-4-(3-isothiocyantopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyantopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150  $\times$  30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984, 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500  $\times$  20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 60F<sub>254</sub> TLC plate eluted with chloroform DBD-F has R<sub>f</sub> 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmeth-



yl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 µL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).

---

#### HPLC VARIABLES

**Column:** 150 × 4.6 µm Inertsil ODS-80A

**Mobile phase:** MeCN:water:trifluoroacetic acid 37:63:0.1

**Column temperature:** 40

**Flow rate:** 1

**Detector:** F ex 460 em 550

---

#### CHROMATOGRAM

**Retention time:** 37.2, 40.0 (enantiomers)

**Limit of detection:** 0.00303-0.00328 fmoles

---

#### KEY WORDS

derivatization; chiral

---

#### REFERENCE

Toyo'oka, T.; Toriumi, M.; Ishii, Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, 15, 1467-1476.